

WHAT IS CLAIMED IS:

1. A conjugate comprising one or more bioactive components covalently attached to at least one linear or branched polyalkylene glycol, wherein said polyalkylene glycol does not comprise an alkoxyl group at any terminus and said polyalkylene glycol is attached to a single bioactive component at a single site on the polyalkylene glycol.

2. The conjugate of claim 1, wherein said conjugate is reduced or substantially reduced in antigenicity compared to a conjugate comprising the same bioactive component(s) linked at the same site or sites on the bioactive component(s) to the same number of polyalkylene glycols of the same size and linear or branched structure containing one or more terminal alkoxyl groups.

3. The conjugate of claim 1, wherein said linear or branched polyalkylene glycol is selected from the group consisting of a poly(ethylene glycol) and a copolymer of ethylene oxide and propylene oxide.

4. The conjugate of claim 3, wherein said linear or branched polyalkylene glycol is a poly(ethylene glycol) ("PEG").

5. The conjugate of claim 1, wherein the attachment of said polyalkylene glycol to said bioactive component(s) is carried out using a reactive derivative of at least one polyalkylene glycol selected from the group consisting of linear dihydroxyPEGs ("PEG diols"), hydroxyPEG-monoacetals and hydroxyPEG-monoacids.

6. The conjugate of claim 1, wherein the attachment of said polyalkylene glycol to said bioactive component(s) is carried out using a reactive derivative of hydroxyPEG selected from the group consisting of a monoaldehyde, a monoester of a monoacid, a monoamine, a monothiol, a monodisulfide, a monobromophenyl carbonate, a monochlorophenyl carbonate, a monofluorophenyl carbonate, a mononitrophenyl carbonate, a monocarbonylimidazole, a monohydrazide, a monocarbazate, a moniodoacetamide, a monomaleimide, a monoorthopyridyl disulfide, a

monooxime, a monophenyl glyoxal, a monothiazolidine-2-thione, a monothioester, a monotriazine and a monovinylsulfone.

7. The conjugate of claim 1, wherein said polyalkylene glycol has a molecular weight of from about 1,000 Daltons (1 kDa) to about 100,000 Daltons (100 kDa).

8. The conjugate of claim 7, wherein said polyalkylene glycol has a molecular weight of from about 2 kDa to about 60 kDa.

9. The conjugate of claim 8, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 2 kDa to about 30 kDa.

10. The conjugate of claim 9, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 5 kDa to about 20 kDa.

11. The conjugate of claim 8, wherein said polyalkylene glycol has a molecular weight of from about 10 kDa to about 20 kDa.

12. The conjugate of claim 11, wherein said polyalkylene glycol has a molecular weight of about 12 kDa.

13. The conjugate of claim 8, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 60 kDa.

14. The conjugate of claim 13, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 22 kDa.

15. The conjugate of claim 14 wherein said polyalkylene glycol has a molecular weight of about 20 kDa.

16. The conjugate of claim 13, wherein said polyalkylene glycol has a molecular weight of about 27 kDa to about 33 kDa.

17. The conjugate of claim 1, wherein said conjugate comprises from about one to about 100 strands of said polyalkylene glycol.

18. The conjugate of claim 17, wherein said conjugate comprises from about one to about five strands of said polyalkylene glycol.

19. The conjugate of claim 18, wherein said conjugate comprises about one or about two strands of said polyalkylene glycol.

20. The conjugate of claim 17, wherein said conjugate comprises from about five to about 100 strands of said polyalkylene glycol.

21. The conjugate of claim 1, wherein said polyalkylene glycol is selected from the group consisting of a monohydroxyPEG-acid and a dihydroxyPEG-acid, such as dihydroxyPEG-lysine.

22. The conjugate of claim 1, wherein said polyalkylene glycol, if linear, has a hydroxyl group at the terminus that is not attached to the bioactive component(s) ("the distal terminus") or, if branched, has a hydroxyl group at every distal terminus.

23. The conjugate of claim 5, wherein said polyalkylene glycol is a reactive derivative of said linear dihydroxyPEG.

24. The conjugate of claim 5, wherein said polyalkylene glycol is a reactive derivative of said hydroxyPEG-monocarboxylic acid.

25. The conjugate of claim 1, wherein said bioactive component is selected from the group consisting of a peptide, a protein, a glycoprotein, an organic compound, an amine-containing compound, a carboxyl-containing compound, a hydroxyl-containing compound and a thiol-containing compound.

26. The conjugate of claim 25, wherein said bioactive component is selected from the group consisting of a peptide, a protein and a glycoprotein.

27. The conjugate of claim 26, wherein said peptide or protein or glycoprotein is selected from the group consisting of an enzyme, a serum

protein, a serum glycoprotein, a blood cell protein, a pigmentary protein, hemoglobin, a viral protein, a peptide hormone, a protein hormone, a glycoprotein hormone, a hypothalamic releasing factor, a cytokine, a growth factor and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing group.

28. The conjugate of claim 27, wherein said serum protein is selected from the group consisting of an albumin, an immunoglobulin, a blood clotting factor and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing serum proteins.

29. The conjugate of claim 27, wherein said peptide hormone or protein hormone or glycoprotein hormone is selected from the group consisting of an antidiuretic hormone, chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone, insulin, prolactin, a somatomedin, growth hormone, thyroid-stimulating hormone, a placental lactogen and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing hormones.

30. The conjugate of claim 27, wherein said growth factor is selected from the group consisting of a colony-stimulating factor, an epidermal growth factor, a fibroblast growth factor, an insulin-like growth factor, a transforming growth factor, a platelet-derived growth factor, a nerve growth factor, a hepatocyte growth factor, a neurotrophic factor, a ciliary neurotrophic factor, a brain-derived neurotrophic factor, a glial-derived neurotrophic factor or a bone morphogenic peptide and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing growth factors.

31. The conjugate of claim 27, wherein said cytokine is selected from the group consisting of erythropoietin, a lymphokine, an interleukin, an interferon, a tumor necrosis factor, a leukemia inhibitory factor and thrombopoietin, and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing cytokines.

32. The conjugate of claim 27, wherein said enzyme is selected from the group consisting of a carbohydrate-specific enzyme, a proteolytic enzyme,

an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase and a ligase.

33. The conjugate of claim 32, wherein said oxidoreductase enzyme is a uricase.

34. The conjugate of claim 32, wherein said proteolytic enzyme is a plasminogen activator.

35. The conjugate of claim 26, wherein said peptide, protein or glycoprotein is an allergen.

36. The conjugate of claim 1, wherein said bioactive compound is a taxane or a derivative thereof.

37. The conjugate of claim 1, wherein said bioactive compound is an antibiotic or a derivative thereof.

38. A pharmaceutical composition comprising the conjugate of claim 1 and a pharmaceutically acceptable excipient or carrier.

39. A method of preventing, diagnosing, or treating a physical disorder in an animal, comprising the administration to said animal of an effective amount of the conjugate of claim 1 or the composition of claim 38.

40. The method of claim 39, wherein said animal is a mammal.

41. The method of claim 40, wherein said mammal is a human.

42. The method of claim 39, wherein said physical disorder is selected from the group consisting of a cancer, arthritis, an infectious disease, a genetic disorder, a neurological disorder, a metabolic disorder, an enzymatic disorder, a cardiovascular disease and hypertension.

43. The method of claim 42, wherein said cancer is selected from the group consisting of a breast cancer, a uterine cancer, an ovarian cancer, a prostate cancer, a testicular cancer, a lung cancer, a leukemia, a lymphoma, a colon cancer, a gastrointestinal cancer, a pancreatic cancer, a bladder cancer, a

kidney cancer, a bone cancer, a neurological cancer, a head and neck cancer, a skin cancer and other carcinomas, sarcomas, adenomas and myelomas.

44. The method of claim 42, wherein said infectious disease is selected from the group consisting of a bacterial disease, a fungal disease, a viral disease and a parasitic disease.

45. The method of claim 44, wherein said viral disease is selected from the group including HIV/AIDS and hepatitis.

46. The method of claim 42, wherein said genetic disorder is selected from the group consisting of amyotrophic lateral sclerosis, cystic fibrosis, Gaucher's disease, hemophilia and other inherited blood disorders, Pompe's disease and severe combined immunodeficiency disease ("SCID").

47. The method of claim 42, wherein said neurological disorder is selected from the group including Alzheimer's disease and multiple sclerosis.

48. The method of claim 39, wherein said administration is parenteral.

49. The method of claim 48, wherein said parenteral administration is intravenous.

50. The method of claim 39, wherein said administration is oral.

51. The method of claim 39, wherein said administration is topical.

52. The method of claim 39, wherein said administration is by inhalation.

53. The method of claim 39, wherein said administration is rectal.

54. A method of producing a conjugate between a bioactive compound and a polyalkylene glycol activated at only one terminus ("a monoactivated polyalkylene glycol"), comprising:

- (a) obtaining a polyalkylene glycol that does not contain any end group that is a stably linked alkoxyl group;
- (b) optionally, prior to the conversion of the polyalkylene glycol of

- step (a) to a monofunctionally activated polyalkylene glycol, protecting all except one of the end groups by the addition of one or more removable blocking groups, such as *t*-butoxyl group(s), aryloxyl group(s) or triphenylmethyl group(s) ("trityl group(s)");
- (c) producing a monofunctionally activated derivative of said polyalkylene glycol by reacting said polyalkylene glycol with a derivatizing compound or compounds under conditions such that said polyalkylene glycol is derivatized with a single derivatizing group at an end that does not contain said removable blocking group or groups;
 - (d) if a blocking group was added to protect the end group(s), as described in step (b) above, removing said blocking group, in one or more steps, without removing the activating group attached as described in step (c) above, to produce a monofunctionally activated polyalkylene glycol wherein the distal terminus or distal termini are hydroxyl groups; and
 - (e) contacting said monofunctionally activated polyalkylene glycol with at least one bioactive component, under conditions that favor the covalent binding of said monofunctionally activated polyalkylene glycol to said bioactive component, or
 - (f) Alternatively, performing step (e) prior to performing step (d).

55. The method of claim 54, wherein said derivatizing group is selected from the group consisting of an aldehyde and a carboxyl group.

56. The method of claim 54, wherein said blocking group is selected from the group consisting of a trityl group, an aryloxyl group and a *t*-butoxyl group.

57. A method for separating a linear monohydroxyPEG-monoaldehyde from the corresponding PEG-dialdehyde comprising:

- (a) converting all hydroxyl groups on the PEG-aldehyde to trityl derivatives;

- (b) separating monotritylPEG-monoaldehyde from the PEG-dialdehyde and any ditritylPEG by reversed-phase chromatography, and
- (c) converting the monotritylPEG-monoaldehyde to mono-hydroxyPEG-monoaldehyde by hydrolytic removal of the trityl group in an acidic medium.

58. The method of claim 57, wherein said aldehyde is, or said dialdehydes are, in the form of acetal derivatives.

59. A conjugate produced by the method of claim 54.

60. The conjugate of claim 59, wherein said conjugate is reduced or substantially reduced in antigenicity compared to a conjugate comprising the same bioactive component linked at the same site or sites on the bioactive component to the same number of molecules of polyalkylene glycol of the same size and linear or branched structure that contain an alkoxyl group at the distal terminus, if the polyalkylene glycol is linear, or contain two or more alkoxyl groups at the distal termini, if the polyalkylene glycol is branched.

61. The conjugate of claim 59, wherein said polyalkylene glycol is selected from the group consisting of a poly(ethylene glycol) and a copolymer of ethylene oxide and propylene oxide.

62. The conjugate of claim 59, wherein the polyalkylene glycol component is selected from the group consisting of a linear poly(ethylene glycol) and a branched poly(ethylene glycol).

63. The conjugate of claim 59, wherein each said polyalkylene glycol has a molecular weight of from about 1 kDa to about 100 kDa.

64. The conjugate of claim 63, wherein said polyalkylene glycol has a molecular weight of from about 2 kDa to about 60 kDa.

65. The conjugate of claim 64, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 2 kDa to about 30 kDa.

66. The conjugate of claim 65, wherein said polyalkylene glycol has two branches, each with a molecular weight of from about 5 kDa to about 20 kDa.

67. The conjugate of claim 64, wherein said polyalkylene glycol has a molecular weight of from about 10 kDa to about 20 kDa.

68. The conjugate of claim 67, wherein said polyalkylene glycol has a molecular weight of about 12 kDa.

69. The conjugate of claim 64, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 60 kDa.

70. The conjugate of claim 69, wherein said polyalkylene glycol has a molecular weight of from about 18 kDa to about 22 kDa.

71. The conjugate of claim 70, wherein said polyalkylene glycol has a molecular weight of about 20 kDa.

72. The conjugate of claim 69, wherein said polyalkylene glycol has a molecular weight of about 27 kDa to about 33 kDa.

73. The conjugate of claim 59, wherein said conjugate comprises from one to about 100 strands of said polyalkylene glycol.

74. The conjugate of claim 73, wherein said conjugate comprises from about one to about five strands of said polyalkylene glycol.

75. The conjugate of claim 74, wherein said conjugate comprises about one or about two strands of said polyalkylene glycol.

76. The conjugate of claim 73, wherein said conjugate comprises about five to about 100 strands of said polyalkylene glycol.

77. The conjugate of claim 59, wherein the monofunctionally activated polyalkylene glycol used in the synthesis of said conjugate is selected from the group consisting of a hydroxyPEG-monoaldehyde and a reactive ester of a hydroxyPEG-monoacid.

78. The conjugate of claim 59, wherein the monofunctionally activated polyalkylene glycol used in the synthesis of said conjugate has a hydroxyl group at its distal terminus, if it is linear, or has a hydroxyl group at every distal terminus, if it is branched.

79. The conjugate of claim 59, wherein the monofunctionally activated polyalkylene glycol used in its synthesis is derived from a linear dihydroxyPEG.

80. The conjugate of claim 59, wherein the bioactive component is selected from the group consisting of a peptide, a protein, a glycoprotein, an organic compound, an amine-containing compound, a carboxyl-containing compound, a hydroxyl-containing compound and a thiol-containing compound.

81. The conjugate of claim 80, wherein said bioactive component is selected from the group consisting of a peptide, a protein and a glycoprotein.

82. The conjugate of claim 81, wherein said peptide or protein or glycoprotein is selected from the group consisting of an enzyme, a serum protein, a serum glycoprotein, a blood cell protein, a pigmentary protein, hemoglobin, a viral protein, a peptide hormone, a protein hormone, a glycoprotein hormone, a hypothalamic releasing factor, a cytokine, a growth factor and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing group.

83. The conjugate of claim 82, wherein said serum protein is selected from the group consisting of an albumin, an immunoglobulin, a blood-clotting factor and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing serum proteins.

84. The conjugate of claim 82, wherein said peptide hormone or protein hormone or glycoprotein hormone is selected from the group consisting of an antidiuretic hormone, chorionic gonadotropin, luteinizing hormone, follicle-stimulating hormone, insulin, prolactin, a somatomedin, growth hormone, thyroid-stimulating hormone, a placental lactogen and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing hormones.

85. The conjugate of claim 82, wherein said growth factor is selected from the group consisting of a colony-stimulating factor, an epidermal growth factor, a fibroblast growth factor, an insulin-like growth factor, a transforming growth factor, a platelet-derived growth factor, a nerve growth factor, a hepatocyte growth factor, a neurotrophic factor, a ciliary neurotrophic factor, a brain-derived neurotrophic factor, a glial-derived neurotrophic factor or a bone morphogenic peptide and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing growth factors.

86. The conjugate of claim 82, wherein said cytokine is selected from the group consisting of erythropoietin, a lymphokine, an interleukin, an interferon, a tumor necrosis factor, a leukemia inhibitory factor and thrombopoietin, and peptides and proteins and glycoproteins that mimic or function as antagonists of any of the foregoing cytokines.

87. The conjugate of claim 82, wherein said enzyme is selected from the group consisting of a carbohydrate-specific enzyme, a proteolytic enzyme, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase and a ligase.

88. The conjugate of claim 87, wherein said oxidoreductase enzyme is a uricase.

89. The conjugate of claim 87, wherein said proteolytic enzyme is a plasminogen activator.

90. The conjugate of claim 81, wherein said peptide, protein or glycoprotein is an allergen.

91. The conjugate of claim 59, wherein the bioactive compound is a taxane or a derivative thereof.

92. The conjugate of claim 59, wherein said bioactive compound is an antibiotic or a derivative thereof.

93. A pharmaceutical composition comprising the conjugate of claim 59 and a pharmaceutically acceptable excipient or carrier.

94. A kit comprising the conjugate of claim 1.

95. A kit comprising the pharmaceutical composition of claim 38.

96. A kit comprising the conjugate of claim 59.

97. A PEG-liposome composition, wherein the PEG component does not comprise an alkoxyl group at any terminus and each molecule of PEG is attached to a single lipid molecule at a single site on the lipid molecule and on said molecule of PEG.

98. The composition of claim 97, wherein said site of attachment is the amino group of a phosphatidyl ethanolamine.

99. The composition of claim 97, wherein said site of attachment is the hydroxyl group of a diacylglycerol.

100. The composition of claim 97, wherein said composition is reduced or substantially reduced in immunoreactivity compared to a PEG-liposome composition comprising at least one alkoxyPEG or a PEG that is attached to a lipid at more than one site or to more than one lipid molecule.